Hereditas 127: 231–238 (1997)

# Spontaneous rate of sister chromatid exchanges (SCEs) in mitotic chromosomes of sheep (*Ovis aries* L.) and comparison with cattle (*Bos taurus* L.), goat (*Capra hircus* L.) and river buffalo (*Bubalus bubalis* L.)

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**Di Berardino, D., Ramunno, L., Jovino, V., Pacelli, C., Lioi, M. B., Scarfi, M. R. and Burguete, I. 1997.** Spontaneous rate of sister chromatid exchanges (SCEs) in mitotic chromosomes of sheep (*Ovis aries* L.) and comparison with cattle (*Bos taurus* L.), goat (*Capra hircus* L.) and river buffalo (*Bubalus bubalis* L.). — *Hereditas 127*: 231–238. Lund, Sweden. ISSN 0018-0661. Received March 27, 1997. Accepted August 8, 1997

The spontaneous level of sister chromatid exchanges (SCEs) in the sheep, estimated by exposing peripheral blood lymphocytes in 0.1 μg/ml of 5′-bromodeoxyuridine (BrdU), was 4.08 ± 2.47 SCE/cell, 2.04 SCE/cell cycle, 0.038 SCE/chromosome. The dose-response relationships, observed by exposing the cells to 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 μg/ml of BrdU, rose rapidly from 0.1 to 0.25 μg/ml, and less rapidly at higher concentrations, thus reaching a saturation level. The analysis of variance, performed on the square root transformed data at 0.1 and 5 μg/ml of BrdU, indicated significant differences ($P < 0.001$) among the four donors tested. The distribution of the SCE/cell frequencies in the cell population of the four donors followed the Poisson 'mixture' probability function, thus confirming previous findings. The spontaneous rate of SCE/cell of sheep is compared with those previously reported for cattle, goat and river buffalo. The theoretical and practical implications of the spontaneous sister chromatid exchanges are discussed in relation to their possible use in animal production for (a) better genetic evaluation of the breeding animals under selection, (b) more precise monitoring of the genotoxic effects of environmental pollutants.

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Sister chromatid exchanges (SCEs) are considered a sensitive test for detecting chromosomal instability syndromes (CHAGANTI et al. 1974) and genetic damage induced by environmental mutagens (CARRANO et al. 1978; LATT et al. 1981). Exchanges are commonly shown after two rounds of DNA replication in the presence of the base analogue 5′-bromodeoxyuridine (BrdU) at final concentations varying from 5 to 20 μg/ml. Since BrdU is itself an SCE inducer (LATT 1973; KLINGERMAN et al. 1982), the exchanges observed under these dosages have to be considered mostly 'induced' by the analogue.

Exchanges, however, are also known to occur spontaneously, as an integral part of the DNA replication process, probably related to error-correcting mechanisms (VERLY et al. 1973; KATO 1974). This fact has important theoretical and practical implications. Spontaneous rates greater than zero mean that SCEs are a normal process during DNA replication, whereas the baseline frequency provides an indication of the extent of somatic recombination occurring in untreated cells (TUCKER et al. 1986).

Detection of the spontaneous rate (or baseline level) of sister chromatid exchanges in domestic animals is, in our opinion, an important task for (a) detecting genetically 'unstable' genotypes, (b) monitoring the genetic damage induced by environmental mutagens.

Since most SCE studies on domestic animals have been carried out by using final concentrations of BrdU between 0.5 and 20 μg/ml (clearly inducers), we decided to estimate the spontaneous rate of SCEs and to characterize the dose/response relationships as well by going down to 0.1 μg/ml of BrdU, which we found to be the limit, in our experimental conditions, for discriminating sister chromatid differential staining.

Previous studies on the description of SCEs in sheep were reported by MCFEE and SHERRILL (1979) and by DUNKELBERG and KRAMES (1984), who used BrdU concentrations between 0.5 and 20 μg/ml.

Specific studies on the detection of spontaneous rate of SCEs have already been reported in cattle (DI BERARDINO et al. 1995), goat (DI BERARDINO et al. 1996), and river buffalo (DI BERARDINO et al., in press). The present study refers to: (a) the estimation of the spontaneous rate of sister chromatid exchanges (SCEs) and BrdU dose/response relationships in sheep (*Ovis aries* L., $2n = 54$); (b) the comparison

with cattle, goat, and river buffalo, under the same experimental conditions.

## MATERIALS AND METHODS

Peripheral blood was aseptically collected from the jugular vein of four sheep donors (2 males and 2 females) of Comisana breed, reared on a farm located in the province of Caserta. The animals were clinically healthy, unrelated, and one year of age. The blood culture technique was the same as previously reported for cattle, goat, and river buffalo.

Briefly, 0.5 ml aliquots of blood ($3 \times 10^6$ lymphocytes) were added to each of six culture flasks containing 9.5 ml of RPMI 1640 medium (Gibco, Dutch modification, New York, USA) including 1 ml of fetal calf serum (Gibco), 0.1 ml of 1-glutamine, 30 µl of antibiotic-antimycotic mixture (Gibco), and 0.1 ml of pokeweed mitogen (Gibco). All cultures were grown at 37.5°C.

After 36 h from initiation, BrdU (Sigma, Saint Louis, MO, USA) was added to each culture flask at final concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, and 5 µg/ml, respectively. The cultures were protected from the light and allowed to grow for additional 36 h. Colcemide was added for the final 30 min (0.05 µg/ml, fixed concentration).

Harvested cells were treated with hypotonic solution and fixed three times with methanol-acetic acid solution 3:1. Air dried slides were stained with a 0.2 % acridine orange solution in phosphate buffer (pH = 7.0) and sealed with paraffin.

SCEs were counted on 50 metaphase spreads in second division, randomly scored for each animal, for each BrdU level. To avoid possible individual bias, all scoring was performed by the same person. In our experimental conditions it was not possible to utilize BrdU doses lower than 0.1 µg/ml because of the poorly defined sister chromatid differential.

## RESULTS

The individual mean rates and standard deviations of the SCEs per cell scored at each BrdU level are reported in Table 1. At the lowest dose of 0.1 µg/ml of BrdU, the individual mean rates of SCE/cell varied from $2.70 \pm 1.65$ to $5.40 \pm 2.24$, with an average of $4.08 \pm 2.47$; at the highest dose of 5 µg/ml of BrdU, the individual SCE/cell rates varied from $6.58 \pm 2.84$ to $9.34 \pm 3.23$, with an average of $7.82 \pm 3.17$.

The individual SCE/cell dose/response relationships from 0.1 to 5 µg/ml of BrdU are shown in Fig. 1. The mean rate increased more rapidly from 0.1 to 0.25 and less rapidly from 0.25 to 5 µg/ml, thus indicating a saturation level.

Table 2 reports the distribution of the SCEs in the cell population of the four animals examined, pooled within each dose of BrdU and tested for fit to the Poisson distribution. Chi-square analysis showed that the simple Poisson expectations did not fit the observations for at least three of the six BrdU dosages, namely, 0.1–0.25 and 1 µg/ml. Only at 0.5 µg/ml was the fit good, while at 2.5 and 5 µg/ml of BrdU the chi-square values were just between 5 and 1 per cent probability levels. However, when at the lowest and highest BrdU dosages, the epexctations were calculated on the basis of the Poisson 'mixture' model, the chi-square values were much lower than those previously found (10.13 versus 52.56 at 0.1 µg/ml, and 8.43 versus 25.96 at 5 µg/ml), thus confirming the better adaptation of the Poisson 'mixture' model, compared with the classical one, to describe the distribution of the SCEs in the cell population.

Fig. 2 illustrates the SCE/cell distributions observed (obs) at 0.1 and 5 µg/ml of BrdU, the Poisson (exp) and the Poisson 'mixture' ($exp_a$) expectations, based on the data reported in Table 2.

Since the SCE frequencies did not follow the normal distribution, the analysis of variance was performed on the square root transformed data $y = (SCE/cell)^{1/2}$, as recommended by previous authors (EREXSON et al. 1983; STEEL and TORRIE 1985; CATALAN et al. 1994) and, for simplicity, only the lowest and the highest BrdU dosages were considered. Significant differences ($P < 0.001$) were found among the four animals examined (Table 3).

The variation in the number and percentage of chromosomes 'without' and 'with' 1, 2 and 3 or more exchanges observed from 0.1 to 5 µg/ml of BrdU is reported in Table 4. By increasing the analogue concentration, the fraction of chromosomes 'without' exchanges decreases significantly ($P < 0.001$) from 92.9 to 86.9 per cent, whereas that of chromosomes 'with' exchanges increases correspondingly from 7.1 to 13.1. Single SCEs are always more frequent (6.7 to 11.6 per cent) compared with double (0.4 to 1.4 per cent) and triple (0.01 to 0.1 per cent) exchanges.

Table 5 reports the variation in the distribution of the SCEs between acrocentric and submetacentric chromosomes: by increasing the BrdU concentration from 0.1 to 5 µg/ml, the fraction of SCEs involving the acrocentrics increases significantly ($P < 0.05$) from 65.4 to 71.9 per cent, while that involving the biarmed chromosomes decreases correspondingly from 34.6 to 28.1 per cent.

Fig. 3 illustrates the BrdU dose-response relationships in the sheep, compared with those previously reported in cattle, goat and river buffalo, under the same experimental conditions.

Table 1. *Sister chromatid exchanges in sheep lymphocytes exposed to varying concentrations of BrdU*[a]

| Donor | BrdU concentration (μg/ml of culture medium) | | | | | |
|---|---|---|---|---|---|---|
| | 0.1 | 0.25 | 0.5 | 1.0 | 2.5 | 5.0 |
| 1 | 5.40 ± 2.24 | 7.26 ± 2.28 | 8.26 ± 2.12 | 8.34 ± 2.92 | 9.16 ± 3.05 | 9.34 ± 3.23 |
| 2 | 5.06 ± 2.45 | 6.74 ± 3.00 | 7.18 ± 2.43 | 7.34 ± 3.28 | 7.92 ± 3.25 | 8.74 ± 2.84 |
| 3 | 2.70 ± 1.65 | 3.82 ± 2.53 | 4.18 ± 1.78 | 5.40 ± 2.35 | 5.48 ± 2.58 | 6.58 ± 2.84 |
| 4 | 3.16 ± 2.34 | 4.60 ± 2.16 | 5.02 ± 2.38 | 5.24 ± 2.54 | 6.14 ± 2.41 | 6.62 ± 2.81 |
| 1–4 | 4.08 ± 2.47 | 5.60 ± 2.88 | 6.16 ± 2.72 | 6.58 ± 3.07 | 7.17 ± 3.17 | 7.82 ± 3.17 |

[a] Each entry is the mean ± SD for 50 metaphase cells

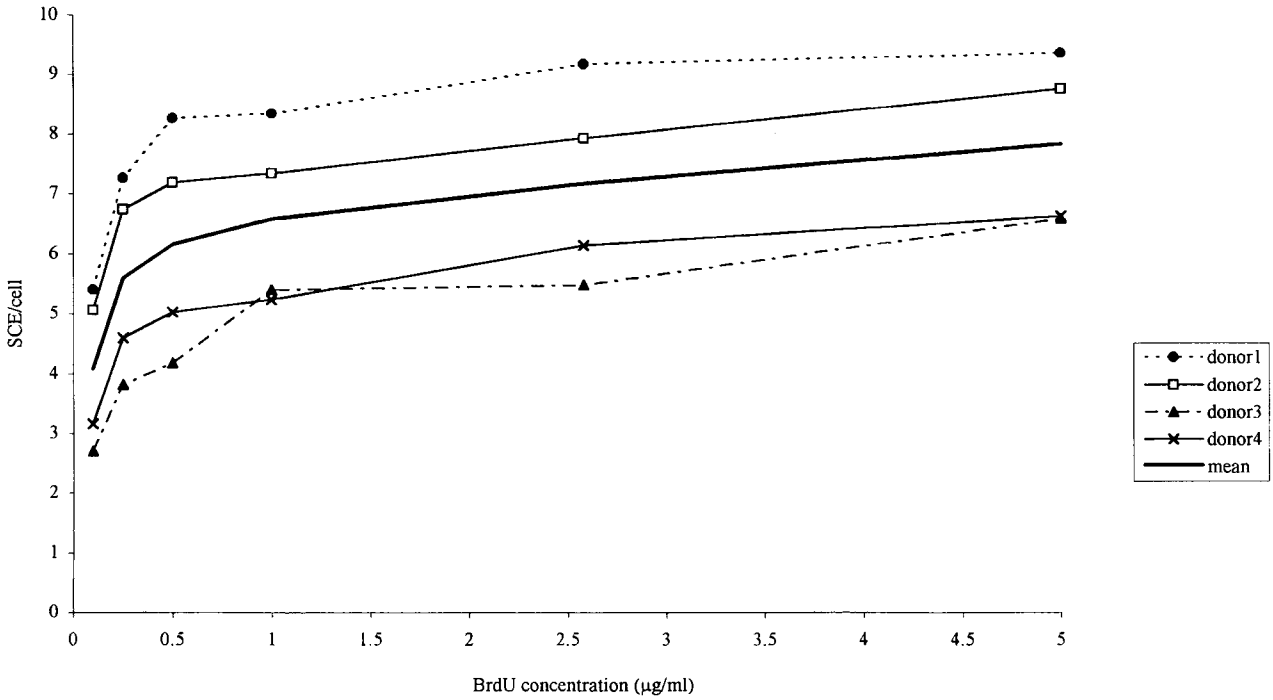


**Fig. 1.** Mean rates of SCE/cell in sheep lymphocytes exposed to increasing doses of BrdU.

The mean rates of 'spontaneous' SCE/cell, SCE/cell generation, and SCE/chromosome observed in the sheep are compared with the corresponding values previously reported for cattle, goat, and river buffalo (Table 6).

## DISCUSSION

The results of the present study indicate that in sheep lymphocytes:

(a) the spontaneous rate of sister chromatid exchanges, estimated at 0.1 μg/ml of BrdU, is 4.08 ± 2.47 SCE/cell, i.e., 2.04 SCE/cell cycle, 0.038 SCE/chromosome. These values are very close to those previously reported in goat, but quite different from cattle and river buffalo.

(b) the BrdU dose/response relationships follow, basically, the same pattern already observed in cattle, goat, and river buffalo, i.e., they increase quite rapidly from 0.1 to 0.25 μg/ml of BrdU, and less rapidly at higher concentrations, thus indicating a saturation level;

(c) the Poisson 'mixture' probability function proved to be a better model, compared with the simple Poisson, for representing the distribution of SCEs in the cell population;

(d) the increased number of exchanges, as a consequence of the increase in the BrdU concentration from 0.1 to 5 μg/ml, was found to involve preferentially the acrocentric chromosomes instead of the submetacentric ones, as previously observed in river buffalo;

Table 2. *Distribution of the observed and expected frequencies of SCE/cell in sheep lymphocytes exposed to varying concentrations of BrdU*[1]

| SCE/cell | BrdU concentration (μg/ml of culture medium) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.1 | | | 0.25 | | 0.5 | | 1.0 | | 2.5 | | 5.0 | | |
| | Obs | Exp | $Exp_a$ | Obs | Exp | Obs | Exp | Obs | Exp | Obs | Exp | Obs | Exp | $Exp_a$ |
| 0 | 9 | 3.38 | 5.64 | 3 | 0.74 | | | | | | | | | |
| 1 | 20 | 13.80 | 18.41 | 11 | 4.14 | 3 | 2.60 | 5 | 1.83 | 3 | 1.10 | 0 | 0 | 0 |
| 2 | 27 | 28.14 | 30.57 | 19 | 11.60 | 15 | 8.02 | 11 | 6.01 | 7 | 3.96 | 6 | 2.46 | 6.00 |
| 3 | 34 | 38.28 | 34.94 | 20 | 21.65 | 20 | 16.46 | 16 | 13.18 | 11 | 9.45 | 6 | 6.40 | 6.65 |
| 4 | 36 | 39.04 | 31.69 | 21 | 30.31 | 23 | 25.34 | 18 | 21.68 | 20 | 16.94 | 17 | 15.52 | 15.88 |
| 5 | 23 | 31.86 | 25.04 | 24 | 33.94 | 22 | 31.22 | 33 | 28.53 | 28 | 24.30 | 22 | 19.57 | 21.18 |
| 6 | 18 | 21.66 | 18.43 | 26 | 31.68 | 32 | 32.06 | 22 | 31.29 | 27 | 29.03 | 28 | 25.51 | 24.00 |
| 7 | 10 | 12.63 | 13.05 | 22 | 25.34 | 23 | 28.21 | 25 | 29.41 | 21 | 29.74 | 23 | 28.50 | 24.01 |
| 8 | 11 | 6.44 | 8.92 | 17 | 17.74 | 23 | 21.72 | 21 | 24.19 | 16 | 26.65 | 23 | 27.86 | 21.91 |
| 9 | 9 | 2.92 | 5.80 | 15 | 11.04 | 13 | 14.87 | 16 | 17.69 | 20 | 21.23 | 13 | 24.21 | 18.76 |
| 10 | 1 | 1.19 | 3.53 | 13 | 6.18 | 11 | 9.16 | 10 | 11.64 | 14 | 15.23 | 19 | 18.93 | 15.39 |
| 11 | 1 | 0.44 | 2.00 | 6 | 3.15 | 9 | 5.13 | 4 | 6.96 | 11 | 9.92 | 14 | 13.46 | 12.26 |
| 12 | 0 | 0.15 | 1.05 | 2 | 1.47 | 5 | 2.63 | 10 | 3.82 | 10 | 5.93 | 11 | 8.77 | 9.49 |
| 13 | 1 | 0.04 | 0.51 | 1 | 0.63 | 0 | 1.25 | 3 | 1.93 | 7 | 3.27 | 9 | 5.28 | 7.12 |
| 14 | | | | | | 1 | 0.55 | 4 | 0.91 | 1 | 1.67 | 5 | 2.95 | 5.13 |
| 15 | | | | | | | | 2 | 0.40 | 2 | 0.80 | 1 | 1.54 | 3.54 |
| 16 | | | | | | | | | | 2 | 0.36 | 3 | 0.75 | 2.33 |
| df | | 12 | | | 12 | | 13 | | 14 | | 15 | | 15 | |
| $\chi^2$ | 52.56 | | 10.13 | | 42.31 | | 18.16 | | 40.07 | | 30.86 | | 25.96 | 8.43 |
| P5 % | | 21.0 | | | 21.0 | | 22.4 | | 23.7 | | 25.0 | | 25.0 | |
| P1 % | | 26.2 | | | 26.2 | | 27.7 | | 29.1 | | 30.6 | | 30.6 | |
| P1 ‰ | | 32.9 | | | 32.9 | | 34.5 | | 36.1 | | 37.7 | | 37.7 | |

[1] For each BrdU level, 200 metaphases are considered. Obs = number of cells observed; Exp = number of cells expected; $Exp_a$ = number of cells expected by considering two subpopulations instead of one.

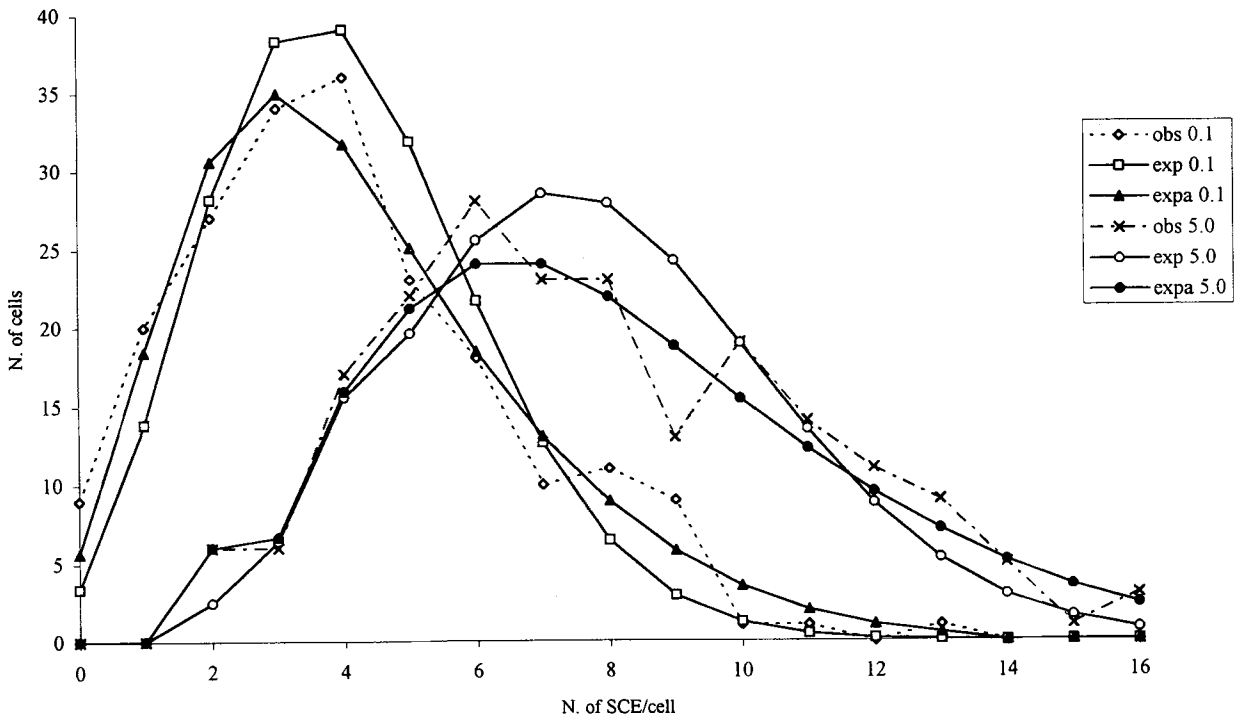


**Fig. 2.** Observed (obs), 'Poisson' expected (exp) and 'Poisson-mixture' expected ($exp_a$) frequencies of SCE/cell at 0.1 and 5.0 μg/ml of BrdU.

Table 3. *Analysis of variance based on square root transformed data ($y = (SCE/cell)^{1/2}$) at 0.1 and 5.0 μg/ml of BrdU*[1]

| BrdU dose | SV | df | MS | F | S |
|---|---|---|---|---|---|
| 0.1 | between | 3 | 5.56 | 19.72 | $P < 0.001$ |
| | within | 196 | 0.28 | | |
| 5.0 | between | 3 | 3.21 | 11.99 | $P < 0.001$ |
| | within | 196 | 0.27 | | |
| 0.1 + 5.0 | donors | 3 | 8.58 | 31.2 | $P < 0.001$ |
| | dosages | 1 | 60.19 | 219.11 | $P < 0.001$ |
| | residual | 392 | 0.275 | | |

[1] SV = Source of variation; df = degrees of freedom; MS = mean square; F = observed statistics; S = significance level

Table 5. *Variation in the distribution of sister chromatid exchanges observed between acrocentric and subhmetacentric chromosomes, from 0.1 to 5.0 μg/ml of BrdU*

| BrdU dose | Total number of exchanges N | Chromosomes | | | |
|---|---|---|---|---|---|
| | | acrocentrics | | submetacentrics | |
| | | N | % | N | % |
| 0.1 | 768 | 502 | 65.4 | 266 | 34.6 |
| 0.25 | 985 | 683 | 69.3 | 302 | 30.6 |
| 0.5 | 1109 | 800 | 72.1 | 309 | 27.9 |
| 1.0 | 1180 | 839 | 71.1 | 341 | 28.9 |
| 2.5 | 1266 | 907 | 71.6 | 359 | 28.3 |
| 5.0 | 1417 | 1019 | 71.9 | 398 | 28.1 |

(e) the differences among the four donors tested were found to be statistically significant both at 0.1 and 5 μg/ml of BrdU, thus confirming the importance of the individual.

*Mean rate of spontaneous SCEs in cattle, goat, sheep, and river buffalo*

The differences among the four species are clearly illustrated in Fig. 3 and in Table 5. The Italian Friesian breed of cattle shows the lowest rate of spontaneous SCE/cell ($2.48 \pm 1.75$), followed by the Ionica breed of goat ($3.28 \pm 1.71$), the Comisana breed of sheep ($4.08 \pm 2.47$), and the river buffalo ($6.66 \pm 3.34$).

Differences in the SCE/cell frequencies are known to depend on a variety of factors such as: concentration and amount of BrdU (LATT 1979; CARRANO et al. 1980), cell proliferation rate (LINDBLAD and LAMBERT 1981), culture conditions (DAS and SHARMA 1983), sex (MARGOLIN and SHELBY 1985), age (HUSUM et al. 1986), dietary habits (WULF et al. 1986), group, animal and treatment factors (CATALAN et al. 1994).

In our experiments, culture conditions were kept constant, the SCE/cell data were averaged between males and females, and only young and clinically healthy animals were used. Therefore, the differences we found among the four species might be explained by other factors, such as (a) karyotype, (b) selection pressure, and (c) evolutionary changes in the AT-GC base composition. Cattle and goat have the same diploid number ($2n = 60$), the same fundamental number (N.F. = 58), the same number of acrocentric autosomes (58) and a remarkable degree of homologies in chromosome banding (ISCNDA 1989) and gene localization (CHOWDHARY et al. 1991; IANNUZZI et al. 1993; HAYES and PETIT 1993; HAYES et al. 1993a and b; POPESCU et al. 1995); the low rate of spontaneous SCE/cell found in the Italian Friesian breed of cattle might be attributed to the high selection pressure that this breed has received, so far, and to the consequent increase in the level of homozygosity. Detection of the spontaneous rate of SCEs on less

Table 4. *Variation in the number and percentage of chromosomes 'without' and 'with' 1, 2, 3 and more exchanges, from 0.1 to 5.0 μg/ml of BrdU*

| BrdU dose | Chromosomes | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 'without' SCEs | | 'with' SCEs | | | | | | | |
| | | | 1 | | 2 | | 3 and more | | 1 + 2 + 3 | |
| | N | % | N | % | N | % | N | % | N | % |
| 0.1 | 10032 | 92.9 | 721 | 6.7 | 46 | 0.4 | 1 | 0.0 | 768 | 7.1 |
| 0.25 | 9815 | 90.9 | 899 | 8.32 | 85 | 0.8 | 1 | 0.0 | 985 | 9.1 |
| 0.5 | 9691 | 89.7 | 991 | 9.2 | 115 | 1.1 | 3 | 0.02 | 1109 | 10.3 |
| 1.0 | 9620 | 89.1 | 1049 | 9.7 | 120 | 1.1 | 9 | 0.08 | 1180 | 10.9 |
| 2.5 | 9534 | 88.3 | 1106 | 10.2 | 145 | 1.3 | 15 | 0.1 | 1266 | 11.7 |
| 5.0 | 9383 | 86.9 | 1259 | 11.6 | 149 | 1.4 | 9 | 0.08 | 1417 | 13.1 |

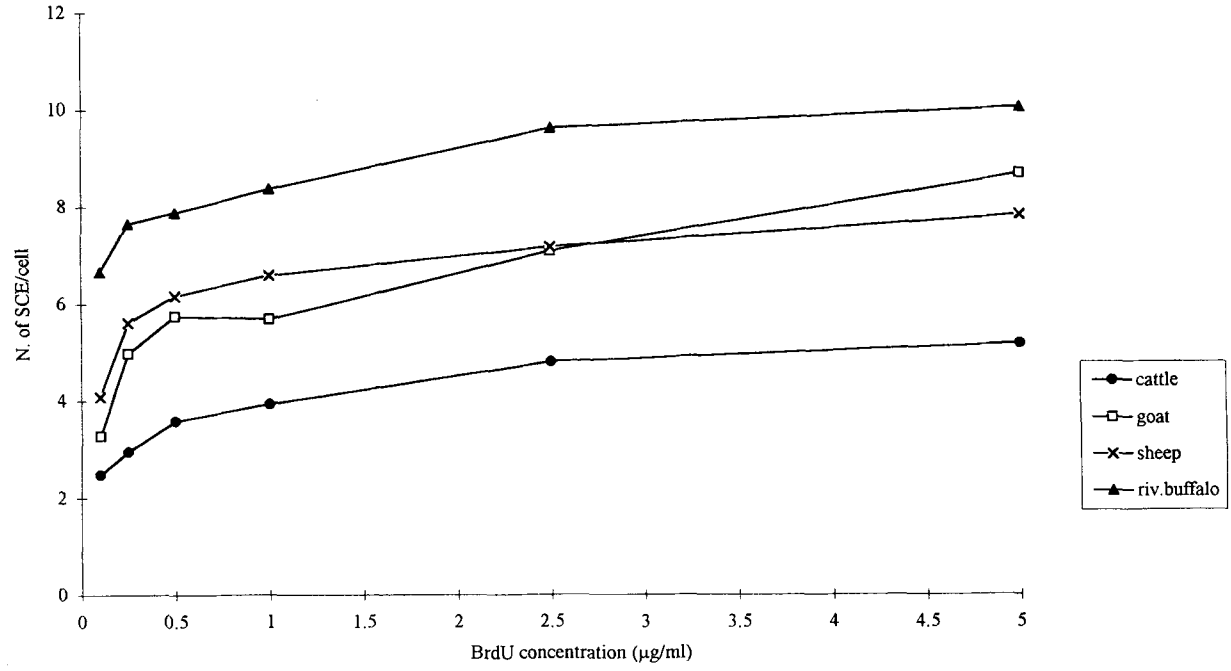


**Fig. 3.** Mean rates of spontaneous SCE/cell and dose/response relationships in cattle, goat, sheep, and river buffalo peripheral lymphocytes exposed to increasing doses of BrdU.

Table 6. *Mean rates of spontaneous SCEs per cell, per cell cycle, and per chromosome, detected at 0.1 μg/ml of BrdU (final concentration) in cattle, goat, sheep, and river buffalo*

| Species | Diploid number | No. of biarmed chromosomes | SCEs | | | Authors |
|---|---|---|---|---|---|---|
| | | | /cell | /cycle | /chromosome | |
| Cattle | 60 | 0 | 2.48 ± 1.7 | 1.24 | 0.020 | Di Berardino et al. 1995 |
| Goat | 60 | 0 | 3.28 ± 1.71 | 1.64 | 0.027 | Di Berardino et al. 1996 |
| Sheep | 54 | 6 | 4.08 ± 2.47 | 2.04 | 0.038 | present study |
| River buffalo | 50 | 10 | 6.66 ± 3.34 | 3.33 | 0.067 | Di Berardino et al., in press |

selected bovine breeds or ecotypes would provide useful information to clarify this problem. On the other hand, goat, sheep, and river buffalo are not exposed to such a selection pressure, mainly because their breeding system is still quite extensive or semi-extensive, zootechnical selection being hampered by basic problems related to ascertaining paternity, the freezing of semen, cyclic oestrus, and so on. Furthermore, since the rate of exchanges is directly related to the amount of BrdU incorporated in A-T rich DNA (Carrano et al. 1980), it is also possible that, during divergence, some homologous autosomes might have been involved in evolutionary changes in the AT-GC base composition. This hypothesis, however, should be further investigated.

The fact that sheep and river buffalo show higher rates of spontaneous SCE/cell compared with goat and cattle might also be accounted for by the presence in their karyotypes of a different number of biarmed chromosomes.

In the sheep karyotype (2n = 54) there are three pairs of biarmed chromosomes, originated by three sets of centric fusions involving acrocentric chromosomes described as nos. 1 and 3 (first pair), 2 and 8 (second pair), and 5 and 11 (third pair) in the standard bovine karyotype (ISCNDA 1989); in the river buffalo karyotype (2n = 50), there are five pairs of biarmed chromosomes originated by five sets of centric fusions between bovine homologous chromosomes nos. 1 and 25 (first pair), 2 and 23 (second pair), 8 and 19 (third pair), 5 and 28 (fourth pair), and 16 and 29 (fifth pair) (Iannuzzi 1994). In Table 6 it is quite evident that the number of biarmed elements in the karyotype might be an important factor, even though previous data (Pathnak et al.

1977) indicated that the SCE/cell rate is, basically, independent of the diploid number.

*BrdU dose-response relationships*

By extrapolating the dose response relationships illustrated in Fig. 3 to 'zero' level of BrdU, the curves intersect the ordinate axis at a value of approximately (i) 2 SCE/cell in cattle and goat, (ii) 3 SCE/cell in sheep, and (iii) 6 SCE/cell in river buffalo. These values could be considered as the real (not estimated) 'baseline' levels of spontaneous SCE/cell for these species.

Interestingly, the curves of the four species examined are all logistic and run parallel to each other, the greatest distance being between river buffalo and cattle. Sheep and goat curves overlap, thus confirming their close evolutionary relationships; indeed, both species belong the the same sub-family Caprinae, sharing also the same sex-chromosome morphology.

Detection of the spontaneous sister chromatid exchanges in domestic animals is of importance because they (a) provide indications about the extent of somatic recombination occurring in untreated cells, (b) allow estimation of the proportion of 'induced' versus 'spontaneous' SCEs. The frequencies of the spontaneous SCEs per cell found in the four species examined are rather high, especially in the river buffalo, if we consider that SGE is an error-prone process (KATO 1974). LYNDAHL and NYBERG (1972) estimated that 2,000 to 10,000 purines would be normally lost from mammalian DNA every cell generation; these apurinic sites have to be repaired continuously by a maintenance system similar to excision repair (VERLY et al. 1973). Spontaneous SCEs might, therefore, be considered as an expression of such a system (KATO 1974). According to this interpretation, differences among species or individuals of the spontaneous rate of SGEs would indicate differences in their repairing efficiency. It would be interesting if this aspect could be verified by in vitro studies of some well known genotoxic DNA-repair inducers. In our opinion, however, such differences should be taken into consideration when domestic animals have to be selected for breeding purposes, in order to avoid the risk to get chromosome aberrations as well as to prevent spreading of 'unwanted' genotypes in the population.

Furthermore, when the SCE test, alone or in addition to chromosomal aberrations, is used for detecting the genotoxic effects of environmental mutagens, it is first necessary to establish the proportion of 'spontaneous' versus 'induced' SCEs; only thereafter can the effect of the mutagen itself be precisely established. Monitoring environmental mutagens is of special importance in animal production because farming areas are known to be quite abundant in pesticides, mycotoxins, and heavy metals. If they reach the food chain, such pollutants represent a continuous risk, not only for animals but also for humans (RUBES 1987).

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